

**Amendments to the Specification:**

Please replace paragraph [0032] beginning at page 6, line 29, with the following:

--[0032] **Figure 8** illustrates a multiple sequencing embodiment of the present invention.

Nucleic acid sequences = SEQ ID NOS:1-4.--

Please replace paragraph [0050] beginning at page 11, line 15, with the following:

--[0050] **FIG. 2** shows a structural model of a PNAC comprising a 9 Degrees North DNA polymerase (parent of Terminator polymerase) 202 and a circular primed DNA template 200. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art will recognize other variations, modifications, and alternatives. The polymerase 202 comprises anchors 203 and 205 inserted at Terminator amino acid positions K53 and K229, respectively. The anchors are identical in amino acid sequence (LLSKKRSLCCXCTVIVYVTDT; SEQ ID NO:5), wherein the anchor comprises amino acid pa-Phe, which is indicated by "X" in the sequence and by white diamonds 204, 206. The pa-Phe amino acids 204, 206 are shown attached to the support 207. The circular DNA template 200 is hybridized to a primer 201. The 5'-end of the primer is indicated 201 and the 3'-end of the primer is hidden in the DNA binding cleft of the protein 202. The structural model is 1QHT.pdb in the protein database at <http://www.rcsb.org/pdb/>.--

Please replace paragraph [0105] beginning at page 28, line 18, with the following:

--[0105] Randomly-sheared fragments of genomic DNA is purified from the sample organism. The DNA is treated with T4 DNA polymerase to generate blunt ends and a single "A" nucleotide

is added to the 3'-ends with Taq DNA polymerase and dATP. A mixture of two double-stranded oligonucleotide adaptors is ligated to the DNA fragments with T4 DNA ligase. *See*, Figures 3-5.

First adaptor (SEQ ID NOS:6 and 7):

Biotin-CGCCACATTACACTTCCTAACACGT  
GCGGTGTAATGTGAAGGATTGTGC

Second adaptor (SEQ ID NOS:8 and 9):

CAGTAGGTAGTCAAGGCTAGAGTCT  
GTCATCCATCAGTTCCGATCTCAG

Ligated DNA products:

genomic DNA: lower case  
adaptors: upper case, (p) 5'-phosphate  
italicized: DNA strand recovered after elution at alkaline pH

Product 1 (SEQ ID NOS:10-13)

Bio-CGCCACATTACACTTCCTAACACGTnnnnn...nnnnnaGACTCTAGCCTTGACTACCTACTGAAA-3'  
GCGGTGTAATGTGAAGGATTGTGCannnnn...nnnnnTCTGAGATCGGAAGTATGGATGACp-5'

Product 2 (SEQ ID NOS:10, 12, 12 and 10, respectively)

Bio-CGCCACATTACACTTCCTAACACGTnnnnn...nnnnnaCGTGTAGGAAGTGTAAATGTGGCG-3'  
3'-GCGGTGTAATGTGAAGGATTGTGCannnnn...nnnnnTGACAATCCTTCACATTACACCGC-Bio

Product 3 (SEQ ID NOS:13, 11, 11 and 13, respectively)

5'-pCAGTAGGTAGTCAAGGCTAGAGTCTnnnnn...nnnnnaGACTCTAGCCTTGACTACCTACTGAAA-3'  
3'-AAAGTCATCCATCAGTTCCGATCTCAGannnnn...nnnnnTCTGAGATCGGAAGTATGGATGACp-5'--

Please replace paragraph [0106] beginning at page 29, line 6, with the following:

--[0106] After ligation, DNA fragments in the size range of about 17-23 kb are purified by gel electrophoresis. The purified fragments are bound to streptavidin-coated magnetic beads (Dyna). After binding, the beads are washed to remove unbound DNA. Then the bound DNA is denatured at alkaline pH and the unbiotinylated strands are eluted (see above; Product 1, italicized font), and the DNA still bound to the beads is discarded. The eluted strands are circularized by hybridization to a primer oligo complementary to both adaptors:

Primed circular template (SEQ ID NOS:14 and 15)

stars mark the ligation site: \*\*

5'-...nnnnnCGTGTAGGAAGTGTAAATGTGGCGCAGTAGGTAGTCAAGGCTAGAGTCTnnnnn...-3' (template strand)  
3'-GCACAATCCTTCACATTACACCGCGTCATCCATCAGTTCCGATCTCAGA-5' (primer)--

Please replace paragraph [0111] beginning at page 30, line 11, with the following:

--[0111] The T7 DNA polymerase gene was amplified from T7 phage DNA using the forward primer

5'-ATGATCGTTTCTGCCATCGCAGCTAAC (SEQ ID NO:16)

(encodes the exonuclease mutations A14-to-C14 and A20-to-C20) and the reverse primer

5'-TCAGTGGCAAATCGCC (SEQ ID NO:17).--

Please replace paragraph [0112] beginning at page 30, line 16, with the following:

--[0112] An oligonucleotide (SEQ ID NO:18) encoding the Strep-Tag II sequence overlapping the 5'-end of the amplified T7 exo-polymerase gene (SEQ ID NO:19) was synthesized on an automated oligonucleotide synthesizer:

5'-ATGTCCAAC TGGTCCCACCCGCAGTTCGAAAAAGGTGGAGGTTC CGCT  
M S N W S H P Q F E K G G G S A  
Strep-Tag II Peptide Spacer

ATGATCGTTTCTGCCATCGCAGCTAAC..

M I V S A I A A N....

T7 polymerase N-terminus overlap (2 exo- mutations underlined)--

Please replace paragraph [0113] beginning at page 30, line 28, with the following:

--[0113] The single-stranded synthetic oligonucleotide was spliced to the amplified T7 gene (above) by overlapping PCR (Horton *et al.* (1989) "Site-directed mutagenesis by overlap extension using the polymerase chain reaction," *Gene* 77:61-68) using the StrepTag forward primer

5'-ATGTCCAACCTGGTCCCACCC (SEQ ID NO:20)

with the reverse primer

5'-TCAGTGGCAAATCGCC (SEQ ID NO:17).--

Please replace paragraph [0122] beginning at page 32, line 24, with the following:

--[0122] A polymerase-coated coverslip is placed on the microscope and a 20  $\mu$ l sample is applied under a water immersion objective lens. The sample contains 40 mM Tris-Cl (pH 7.5), 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, 0.1 mg/ml of bovine serum albumin, 12.5 mM magnesium chloride, 10 nM dUTP-TMR, 100 nM each of dATP, dCTP, and dGTP, and 10  $\mu$ g/ml of primer-template DNA. Depending on the activity of the immobilized enzymes, the nucleotide concentration may have to be adjusted so that individual incorporation events are time-resolvable. Data are collected and analyzed as described in Example 6 to determine whether the dUTP-TMR nucleotide is incorporated into the primer strand. (In order to perform this experiment in a droplet on an open coverslip as described, it may be necessary to speed the motion of free dUTP-TMR through the imaged zone by drive convection with a nitrogen stream, depending on ambient conditions. It is also necessary to use a water immersion objective lens immersed directly in the sample.) The results are compared against a control without primer-template DNA to demonstrate the appearance of longer fluorescence bursts in the test sample indicating a template sequence which supports dUTP incorporation. Two sample primer-templates are compared; they are synthetic oligonucleotides derived from the cystic fibrosis transmembrane conductance regulator gene (Welsh *et al.* (1993), *J. Cell Science* 106S:235-239).

Appl. No. 10/821,689

PATENT

Amdt. dated July 23, 2004

Reply to Notice to File Corrected Application Papers of June 28, 2004

Normal Allele (SEQ ID NOS:21 and 22) (does not incorporate dUTP- $\gamma$ -TMR)

primer 3'-CACCATTAAAGAAAATATCAT

template 5'-GUGGUAAUUUCUUUUAUAGUAG

~~(DELTA)F508 DELETION MUTANT (DOES INCORPORATE DUTP- $\gamma$ -TMR)~~

(Delta)F508 Deletion Mutant (SEQ ID NOS:21 and 23) (does incorporate dUTP- $\gamma$ -TMR)

primer 3'-CACCATTAAAGAAAATATCAT

template 5'-GUGGUAAUUUCUUUUAUAGUAA--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 7, at the end of the application.